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14. ABSTRACT
The hypothesis for this study is that copy number alterations (amplification and deletion) in a limited repertoire of genes Is highly predictive of prostate cancer metastasis. This signature is present in primary prostate cancers at the time of diagnosis and is enriched in the primary prostate cancers of African-American men, thus accounting for the health disparity of prostate cancer metastasis among them. The biological effect of these copy number events is to convey an escape from anoikis, as well as the other features that occur with metastasis. The current study confirmed this signature in prostate cancers that have been shown to metastasize, compared to those that have not and determine the prevalence of this high-risk signature in the prostate cancers of African-American men matched for stage compared to those of European-American men. This study demonstrated that the signature can be detected in prostate cancer biopsies. This study answered an important question about the apparent health disparity of prostate cancer metastasis and developed a clinically useful tool that could be used to select treatment men diagnosed with prostate cancer.

15. SUBJECT TERMS
Prostate cancer, health disparity, metastasis, African-American men, genomics, copy number alteration

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INTRODUCTION

Compared to European-American (EA) men, African-American (AA) men have a 2-fold greater risk of dying from metastatic prostate cancer (1, 2). For both groups, proper categorization of prostate cancer biopsies as high or low-risk for metastasis at the time of diagnosis would optimize treatment, improving outcomes and minimizing toxicity. The Ostrer laboratory has demonstrated that the specific genes within metastatic prostate cancers have been altered by amplification (increase in the copy number) or deletion (decrease in the copy number) (3). These genes appeared to have been selected by the advantages that they conveyed to tumors, such as escape from cell death ('anoikis'). These amplified or deleted metastasis genes are enriched 2.5-fold in the primary prostate cancers of AA men – a degree of enrichment that is similar to the enhanced likelihood of metastasis. The current study was designed to confirm these observations about gene patterns predictive of metastatic potential in new cohorts of men for whom outcome data are available. These methods will be applied to prostate cancer biopsy specimens to demonstrate that they could be used at the time of diagnosis for prediction of outcome. This study will be beneficial to all men with prostate cancer, because it provided a diagnostic tool. With the establishment of a licensing agreement with Affymetrix, this tool can be carried into clinical practice for selection of therapy. It is especially beneficial for African-American men who have a greater likelihood of disease and metastasis.

KEYWORDS

Prostate cancer, metastasis, African-American men, health disparity, genomics, copy number alteration, predictive signature.

ACCOMPLISHMENTS

Major goals

1. Validate and optimize the pipeline/approach for identifying prostate cancer metastatic potential/health disparity study through somatic genomic DNA copy number analysis.

2. Develop a risk model using a comprehensive set of genomic markers.

3. Sequence the exomes of primary and metastatic tumors from African American and Caucasian American men.

Summary of accomplishments

The accomplishments of this study are detailed in the manuscript that was submitted to the *Journal of Clinical Oncology* (Appendix) and are summarized here.

1. We validated a metastasis profile signature (MPS) based on copy number alterations (CNAs) for prediction of metastasis, metastasis-free survival and the surrogate endpoint

of biochemical recurrence in two independent cohorts – one obtained from Duke University and the second obtained from Memorial Sloan-Kettering Cancer Center (MSKCC).

For the outcome of metastasis, univariate logistic regression of MPS resulted in significant AUCs for the MSK (0.70, p =0.001) and the Duke cohorts (0.75, p = 0.002) (Appendix: Table 1 and Fig 1).

The MPS was developed to estimate the risk of metastasis, but was also tested for prediction of BCR. BCR is a necessary, however insufficient, clinical transition point towards the metastasis outcome. All metastases are BCR+, however, only twenty-five percent of BCR+ tumors progress to metastasis. For the outcome of BCR, univariate logistic regression of MPS resulted in significant AUCs for the MSK (0.67 p = 5.5E-05) and the Duke cohorts (0.70 p = 0.003) (Appendix: Table 2).

As a continuous univariate predictor through a Cox model, the MPS was associated with metastasis-free survival in both the MSK (HR = 4.2, p = 0.002) and Duke (HR = 3.9, p = 0.006) cohorts with a concordance index of 0.70 and 0.63, respectively (Appendix: Table 3 and Table S5).

2. We showed that the accuracy of the model was improved by the inclusion of pre-operative PSA, but not by the inclusion of clinical stage, biopsy Gleason, age at diagnosis, nor percent genomic instability. The resulting accuracy of this model is comparable to others that have been reported.

Combining MPS and pre-operative PSA in a multivariate logistic model resulted in an AUC = 0.80 for both cohorts with the MPS reaching statistical significance in both MSK (p = 0.04) and Duke (p = 0.001) cohorts. All other pre-operative clinical variables did not improve the AUC in logistic regression analysis (Appendix: Table S3). In univariate logistic analysis of the MSK cohort, percent genomic instability had an AUC = 0.74, p = 1.4E-05, however, this variable did not reach statistical significance in the Duke cohort (AUC = 0.80, p = 0.12) (Appendix: Fig 1 and Table S2). This indicates that percent genomic instability, while useful in the MSK cohort, is not an independent and robust predictor of metastasis.

Unlike the MPS, in logistic analysis for BCR, percent genomic instability was significant only in the MSK cohort (AUC = 0.65, p = 1.8E-05) (Appendix: Table S4). Adding percent genome instability to MPS did not result in improvement in AUC in either cohort. Of note, pathological Gleason (AUC = 0.68, p = 2.2E-08) and pathological stage (AUC = 0.68, p = 2.0E-06) were predictive of BCR outcome only in the MSK cohort, but not in the Duke cohort, reflecting the matching of mPTs and iPTs in the Duke cohort. All other pre-operative clinical variables did not improve the AUC in logistic regression analysis (Appendix: Table S4).

In a multivariate Cox model, adding pre-operative PSA to the MPS improved the concordance index of both the MSK (0.74) and Duke (0.70) cohorts. In univariate Cox

analysis of the MSK cohort, percent genomic instability was associated with metastasis-free survival (HR = 1.11, p = 3.3E-07; concordance index = 0.67, p = 0.02), as previously reported for this cohort (4); however, this variable did not reach statistical significance in the Duke cohort.

For the outcome of time-to-BCR in both cohorts, MPS achieved statistical significance when applied to a univariate Cox model in the MSK (HR = 3.5, p = 1.5E-05, concordance index of 0.67, p = 1.1E-07) and Duke cohorts (HR = 3.5, p = 0.002, concordance index of 0.62, p = 0.004 – Appendix: Table 4 and Table S6). In a multivariate Cox model that included both MPS and preoperative PSA, MPS was significant only in the MSK cohort (HR = 3.4, p = 2.5E-05, concordance index = 0.69, p = 1.6E-09). Percent genomic instability had a significant association in the MSK cohort (HR = 1.11, p= 5.6E-09, concordance index = 0.65) and a marginal effect size in the Duke cohort. For illustration, the log rank test of the MPS plotted as Kaplan-Meier survival curves (cut at MPS = 1.2 and representing the top 50% the MPS distributions and 35% of the samples) resulted in a significant separation in both Duke (p=0.01, p=0.006) and MSK cohorts (p=0.01, p=0.0004) for the outcomes of metastasis and biochemical recurrence free probability, respectively (Appendix: Fig 2).

3. We observed that the model was equally applicable to the African-American and European-American subjects in the study.

4. We developed a NextGen genotyping with Affymetrix-Eureka Genomics (Affy-EG) that could be used as a clinical test on DNA derived from prostate cancer biopsies. This test quantifies CNAs in 900 genetic markers established in this study to have the most predictive power to estimate the metastatic potential of the tumor. Affy-EG created NGG by combining ligase-mediated (LM)-PCR with next generation sequencing (NGS). The uniformly-sized ligated products are PCR amplified using DNA oligonucleotide primers *uniquely indexed* for each sample. This exceptional indexing feature permits thousands of samples to be condensed into a single library for NGG data generation, thereby containing costs and increasing throughput. The NGG sequence data are tabulated to quantify (count) the number of reads associated with each sample locus. These counts are used by Affy-EG's proprietary genotyping pipeline to produce genotyping or copy number calls and the corresponding confidence levels.

5. Based on our analysis of prostate subjects in the Cancer Genome Atlas, we observed that no somatic mutations occurred with sufficient frequency to improve the accuracy of the MPS PC Amplifier tests.

Opportunities for training and professional development

Nothing to report

Dissemination of results to communities of interest

1. A manuscript was submitted for publication.

2. A clinical test will be commercialized by the licensing partner.

Plans for next reporting period

Nothing to report

IMPACT

Innovation

This study was innovative, because it applied individual genomic data analysis to the prediction of prostate cancer metastasis, the major cause of death from prostate cancer and the second health disparity of prostate cancer (after ethnic risk of disease). Personalized or precision medicine is about applying the right treatment to the right disease at the right time and neither over-treating nor under-treating the patient. A commercialized PC Amplifier test will innovate care by identifying those patients who would benefit from aggressive treatment at the time of diagnosis and those who would be candidates for active surveillance. Thus, although surgery or radiation therapy cures ~70% of prostate cancers and ~90% of men with low-risk disease, only 30% (~5% for low-risk disease) would have gone on to metastasize. Thus, aggressive therapies may simply not be required for the majority of men treated and especially among men with low-risk disease. As such, better risk stratification tools, such as the PC Amplifier test, could select the men unlikely to develop metastases and allow these men to undergo active surveillance. These men could gain confidence that active surveillance was not impairing cancer control while sparing them the ~50% risk of quality of life changing toxicities, including erectile dysfunction, urinary dysfunction and rectal bleeding.

Impact on other disciplines

In a related study, not funded by this grant, we tested the commonality of these CNA events across primary cancers and the validity of this score for predicting risk of metastasis. We identified CNAs on a genomewide basis in a set of 43 triple negative breast cancers with known metastasis outcomes or 5 years or more of metastasis-free survival. We observed high areas (AUCs) under the receiver operator curves for prediction of metastasis using the MPS for triple negative breast cancers. Thus, these findings demonstrate that a series of discrete CNAs are shared across primary tumors of different types, suggesting common mechanisms of metastasis, that scoring these events can be used clinically at the time of diagnosis for predicting outcomes and that the products of these altered genes might be targeted with specific therapies to prevent metastases.

Impact of technology transfer

Implementation of the Affy-EG PC Amplifier test could create a sea of change by predicting a clinically meaningful end-point at low-cost. Introduction of an FDA-approved

PC Amplifier test would improve access to care by creating a plethora of new testing laboratories, unlike the current state of high-cost LDTs offered by sole-source providers, including Myriad Genetics' Prolaris, Genomic Health's OncotypeDx, GenomeDx's Decipher, and MDxHealth's Confirm. As noted in the proposal, the amplified genes present in the MPS may be candidates for targeted therapies, creating new treatment options for high-risk tumors and distant metastases in the future.

Impact on society beyond science and technology

Nothing to report

CHANGES/PROBLEMS

Nothing to Report

PRODUCTS

Journal publications

Pearlman A, Upadhyay K, Cole K, Loke J, Sun K, Freedland SJ, Shao Y & Ostrer H, "Robust Genomic Predictor of Prostate Cancer Metastasis," submitted to *Journal of Clinical Oncology*

Conference papers and presentations

Pearlman A, Campbell C, Loke J, Brooks E, Genshaft A, Shajahan S, Ittmann M, Bova GS, Melamed J, Holcomb I, Schneider RJ, Freedland SJ, Shao Y & Ostrer H, **"**Prostate cancer metastasis prognostic bio-marker development," presented at American Society of Human Genetics annual meeting, November, 2012.

*Pearlman A, Upadhyay K, Cole K, Loke J, Sun K, Campbell C, Freedland SJ, Shao Y & Ostrer H, "Prognostic Prediction of Prostate Cancer Metastasis and Biochemical Recurrence," presented at American Society of Human Genetics annual meeting, October, 2012.

Patent application

"Genomic Signatures of Metastasis in Prostate Cancer" Date of application to U.S. Patent and Trademark Office May 5, 2012

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

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Project Role:	*PI*
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Nearest person month worked:	*2*
Contribution to Project:	*Dr. Ostrer oversaw all aspects of this study – design, recruitment, data generation, analysis, manuscript writing, public presentations, reporting to the funding agency and compliance with research regulations.*
Funding Support:	*Department of Defense* *National Institutes of Health* *Albert Einstein College of Medicine* *Montefiore Medical Center*
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Nearest person month worked:	*4*
Contribution to Project:	*Dr. Pearlman worked on all aspects of this study – design, recruitment, data generation, analysis, manuscript writing, public presentations, reporting to the funding agency and compliance with research regulations.*
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Project Role:	*Statistical consultant*

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Nearest person month worked:	*1*
Contribution to Project:	*Dr. Shao advised about the statistical methods and analyses.*
Funding Support:	
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Nearest person month worked:	*1*
Contribution to Project:	*Dr. Freedland aided with study design, subject recruitment, analysis manuscript writing, and compliance with research regulations.*
Funding Support:	
Name:	*Jennifer Stout*
Project Role:	*Research coordinator*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*3*
Contribution to Project:	*Helped to recruit subjects, collect and process tissue samples*
Funding Support:	
Name:	*Leah Gerber*
Project Role:	*Database manager*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*1*
Contribution to	*Helped abstract and clean clinical data for subjects; helped*

Project:	*in subject identification*
Funding Support:	

Change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period

Nothing to Report

Other organizations involved as partners

Organization Name: Affymetrix-Eureka Genomics
Location of Organization: Hercules, CA
Partner's contribution to the project: Collaboration

SPECIAL REPORTING REQUIREMENTS

Nothing to Report

APPENDIX

Attached

Robust Genomic Predictor of Prostate Cancer Metastasis

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Running head: Metastasis profile signature

ABSTRACT

Purpose The goal of this study was to demonstrate the clinical validity of the metastatic potential score (MPS) to predict the risk that a localized prostate cancer would metastasize and, thus, could be applied pre-operatively.

Patients and Methods Metastatic potential score (MPS) was calculated from radical prostatectomy specimens of 62 men whose tumors metastasized and 181 men whose tumors did not metastasize after at least five years of follow-up in two independent cohorts. Multivariate logistic regression and Cox proportional hazards models were used to assess the accuracy of the MPS and other early pre-operative clinical predictors to estimate metastasis risk.

Results A logistic regression model using the proposed MPS and pre-operative PSA levels as predictors resulted in an 80% area under the ROC curve, $p < 0.01$ for the outcome of metastasis in both cohorts. Accordingly, a Cox regression model using MPS and pre-operative PSA levels as predictors for the outcome of metastasis-free survival resulted in a concordance index of 0.70 and 0.74 for the Duke and MSK cohorts respectively.

Conclusion MPS and pre-operative PSA combine into a robust predictor of metastatic potential in two independent cohorts.

INTRODUCTION

Prostate cancer is the most commonly diagnosed male cancer and the second leading cause of cancer deaths among men, accounting for 9% of male cancer deaths in the United States.[1] As radical prostatectomy or radiation therapy can lead to reduced risk of metastasis, but erectile dysfunction, urinary incontinence, and rectal bleeding may occur in up to 50% of patients, affecting their quality of life.[2,3] Men with clinical low-risk disease as measured by pre-surgical Gleason scores are often candidates for active surveillance that might safely preserve quality of life, but at the risk of allowing an undetected more aggressive cancer to go untreated. Alternatively, men with clinical intermediate and high-risk disease often receive aggressive therapies, even though many are unlikely to die from their disease even in the absence of treatment. Within all of these risk groups, clinical outcomes are varied between indolent disease and more aggressive disease characterized by disease recurrence usually measured as biochemical recurrence – BCR and distant metastasis, despite current treatment approaches. Matching therapy or surveillance to prognosis at the time of diagnosis could improve outcomes and quality of life for men with prostate cancer. Analysis of innate features of these tumors to predict outcome represents one way by which this matching might be accomplished.

Primary tumors are programmed by genetic alterations, including copy number alterations (CNAs), to have varying clinical courses.[4,5] The burden of CNAs at prostatectomy, measured as the percentage of the genome in primary prostate cancers undergoing genetic gains and losses, has been shown to be

associated with BCR and, more recently, with risk of metastasis.[6,7] Recently, our laboratory observed that despite the large size of these CNA regions, 365 genes within these regions were commonly altered in metastases and primary tumors with similar patterns. Many of these genes are known to be correlated with metastasis risk, including SLC7A5,[8] Cadherin family members (CDH2, CDH8, CDH13, CDH15, CDH17 and PCDH9),[9] and potassium channel genes (KCNB2, KCNQ3, KCNAB1, KCTD8 and KCNH4).[10] As a result, a metastasis potential score (MPS) was developed based on the weighted frequency of specific genetic CNAs observed in metastases. As a continuous predictor, applying the MPS to a Cox proportional hazards model resulted in a significant association to the endpoint of metastasis-free survival (2.88; 95%CI = 1.15-7.2; p=0.02) in the initial study with a small number of cases with documented metastasis outcome.[11]

Here, we assess the accuracy and the clinical validity of MPS and other pre-operative predictors in two larger surgical specimen radical prostatectomy cohorts in which long-term prospective outcome information was obtained and compare the accuracy and applications of this method to the recently published method using CNA burden as well as other predictors that have been reported.[12,13]

METHODS

Predictive Biomarkers

This study provides in-depth comparison of existing prostate cancer genomic DNA metastasis signature and methodology used to calculate a MPS

from prostate tumor CNAs,[11] with CNAs used to calculate percent genomic instability.[7] The MPS methodology is platform-independent, but requires that genomic DNA signal intensities are captured within the regions of the metastasis signature. In this study, the analysis was conducted on a primary data set reported here utilizing the Affymetrix Oncoscan FFPE V3 array[12] and on a previously generated data set assayed on Agilent 240K arrays.[7] Percentage of the genome altered by copy number gains and losses ("genomic instability") was calculated by OncoScan™ Nexus Express Software.

Cohorts, tissue and sample processing

A prostate cancer radical prostatectomy cohort of 37 men that progressed to metastasis (mPTs) and 24 men that were BCR-free and metastasis-free (iPTs) after at least five years of follow up was collected at Duke University (Duke cohort - Tables S1 and S2). The Duke cohort had a case-control design that matched mPTs and iPTs for age, race, pathological stage, margin status, Gleason score, and surgery year. BCR was scored as positive or negative, but not matched. Two tumor blocks from each patient were cut into eleven 10um sections. The first and last sections (5um only) were stained with H&E and evaluated by pathologists who reported a similar representation of tumor cells in both sections. The Duke specimens were then sent to Albert Einstein College of Medicine, where they were reviewed by a single pathologist and scored using the 2005 International Society of Urological Pathology Consensus guidelines.[14] Primary and secondary Gleason scores were assigned to the region of the

carcinoma. The area of the tumor region relative to the entire section was estimated by the pathologist. To quantify the extent of tumor nuclei relative to stromal cells in a selected section, digital images of the H&E tumor slides were analyzed with histologic image analysis software (Imagescope) to determine the percent of tumor nuclei within the selected tumor region. Tumor regions were microdissected, extracted for DNA, and assayed on the Oncoscan FFPE V3 array (Affymetrix Oncoscan Service, Santa Clara, California).

A second cohort, comprised of 25 mPTs along with 157 iPTs was collected at Memorial Sloan-Kettering Cancer Center (MSK cohort - Tables S1 and S2). The collection, extraction and data generation for the second cohort has been previously described.[7] The MSK cohort represented a consecutive case-cohort design with BCR-negative and non-metastatic outcome samples making up a disproportionate number. Unlike the Duke samples, these samples were not matched on any criteria. The MSK cohort was comprised of fresh frozen radical prostatectomies. The Duke and MSK cohorts differed in their length of follow-up, clinical and pathologic attributes and BCR and metastasis outcomes (Tables S1 and S2). The Duke cohort was collected for individuals with greater than five years follow-up since the majority of prostate cancers recur via BCR or metastasize within this timeframe. To achieve parity for prediction modeling and maximizing the BCR and metastasis informativeness of each patient, the MSK cohort was filtered for subjects that had at least five years of follow-up. Also, for both cohorts, metastasis negative subjects treated with radical prostatectomy and adjuvant radiation and/or hormonal therapy were excluded from analysis to

provide a more homogeneous iPT group. This study was reviewed and approved by the Institutional Review Boards at Albert Einstein College of Medicine, New York University School of Medicine, and Duke University.

Metastatic Potential Score (MPS)

The MPS was calculated based on genomic CNAs with a higher score indicating a greater likelihood of metastasis.[11] Univariate and multivariate logistic regression and Cox proportional hazards survival models were evaluated for MPS, pre-surgery predictors (PSA, clinical stage, biopsy Gleason), demographic variables (age at diagnosis and race), and percent genomic instability, as described previously for MSK cohort.[7] Area under the receiver operating characteristic curves (ROC-AUC) and concordance index were calculated for the logistic and Cox models, respectively. Kaplan-Meier survival curves were plotted (cut at MPS = 1.2 and representing the top 50% of the MPS distribution and 35% of the samples) and Mantel-Cox log rank test was calculated for the clinical endpoints of time to metastasis and BCR.

RESULTS

Risk of metastases and BCR on logistic regression

For the outcome of metastasis, univariate logistic regression of MPS resulted in significant AUCs for the MSK (0.70, p =0.001) and the Duke cohorts (0.75, p = 0.002) (Table 1 and Fig 1). Combining MPS and pre-operative PSA in a multivariate logistic model resulted in an AUC = 0.80 for both cohorts with the

MPS reaching statistical significance in both MSK (p = 0.04) and Duke (p = 0.001) cohorts. All other pre-operative clinical variables did not improve the AUC in logistic regression analysis (Table S3). In univariate logistic analysis of the MSK cohort, percent genomic instability had an AUC = 0.74, p = 1.4E-05, however, this variable did not reach statistical significance in the Duke cohort (AUC = 0.80, p = 0.12) (Fig 1 and Table S2). This indicates that percent genomic instability, while useful in the MSK cohort, is not an independent and robust predictor of metastasis.

The MPS was developed to estimate the risk of metastasis, but was also tested for prediction of BCR. BCR is a necessary, however insufficient, clinical transition point towards the metastasis outcome. All metastases are BCR+, however, only twenty-five percent of BCR+ tumors progress to metastasis. For the outcome of BCR, univariate logistic regression of MPS resulted in significant AUCs for the MSK (0.67 p = 5.5E-05) and the Duke cohorts (0.70 p = 0.003) (Table 2). Unlike the MPS, in logistic analysis for BCR, percent genomic instability was significant only in the MSK cohort (AUC = 0.65, p = 1.8E-05) (Table S4). Adding percent genome instability to MPS did not result in improvement in AUC in either cohort. Of note, pathological Gleason (AUC = 0.68, p = 2.2E-08) and pathological stage (AUC = 0.68, p = 2.0E-06) were predictive of BCR outcome only in the MSK cohort, but not in the Duke cohort, reflecting the matching of mPTs and iPTs in the Duke cohort. All other pre-operative clinical variables did not improve the AUC in logistic regression analysis (Table S4).

Cox proportional hazards analysis

As a continuous univariate predictor through a Cox model, the MPS was associated with metastasis-free survival in both the MSK (HR = 4.2, p = 0.002) and Duke (HR = 3.9, p = 0.006) cohorts with a concordance index of 0.70 and 0.63, respectively (Table 3 and Table S5). In a multivariate Cox model, adding pre-operative PSA to the MPS improved the concordance index of both the MSK (0.74) and Duke (0.70) cohorts. In univariate Cox analysis of the MSK cohort, percent genomic instability was associated with metastasis-free survival (HR = 1.11, p = 3.3E-07; concordance index = 0.67, p = 0.02), as previously reported for this cohort;[7] however, this variable did not reach statistical significance in the Duke cohort.

For the outcome of time-to-BCR in both cohorts, MPS achieved statistical significance when applied to a univariate Cox model in the MSK (HR = 3.5, p = 1.5E-05, concordance index of 0.67, p = 1.1E-07) and Duke cohorts (HR = 3.5, p = 0.002, concordance index of 0.62, p = 0.004 – Table 4 and Table S6). In a multivariate Cox model that included both MPS and preoperative PSA, MPS was significant only in the MSK cohort (HR = 3.4, p = 2.5E-05, concordance index = 0.69, p = 1.6E-09). Percent genomic instability had a significant association in the MSK cohort (HR = 1.11, p= 5.6E-09, concordance index = 0.65) and a marginal effect size in the Duke cohort. For illustration, the log rank test of the MPS plotted as Kaplan-Meier survival curves (cut at MPS = 1.2 and representing the top 50% the MPS distributions and 35% of the samples) resulted in a significant separation in both Duke (p=0.01, p=0.006) and MSK cohorts (p=0.01,

p=0.0004) for the outcomes of metastasis and biochemical recurrence free probability, respectively (Fig 2).

Tumor Heterogeneity

Intra-tumor variation of CNA gains or losses may be a function of assay variability and/or proportion of normal genomic DNA contamination. Alternatively, variation in CNAs can emerge from different clones from the same patient's tumor. To assess the prevalence of tumor heterogeneity, twenty-two pairs of tumors dissected from two distinct tumor blocks from twelve patients were analyzed. Calculating the MPS difference between the series of paired tumors resulted in two out twenty two tumor pairs (9%) exhibiting multi-clonal genomic profiles (Fig. S1). For samples with very low genomic instability (< 1%), ≥10 percent tumor nuclei of the H&E stained sections were used to proceed with an MPS analysis.

DISCUSSION

These findings demonstrate that the previously reported MPS based on analyzing specific CNA events in the tumor genome is a robust test for assessing risk of metastasis at the time of diagnosis or following surgery.[11] Despite the variation in design, the accuracy of MPS plus pre-operative PSA as measured by AUC was virtually identical in these two cohorts (~80%). MPS was the only significant predictor in univariate analyses in both cohorts. The accuracy of prediction was improved for both outcomes by inclusion of pre-operative PSA.

MPS was a more robust predictor of metastasis, metastasis-fee survival and BCR than the previously reported CNA burden test in two different surgical cohorts, including the cohort from which the CNA burden test was developed.[7] Although percent genomic instability has been proposed as an alternative measure for predicting BCR and metastasis, it was not a significant predictor for either outcome in the Duke cohort. The improvement of MPS over CNA burden is contributed by quantifying specific genomic events that are associated with disease progression.

The accuracy of combined MPS and pre-operative PSA predictor appears to be similar to the various RNA expression profile tests plus clinical predictors for use as a post-surgical tool (Table S7). These tests, Genomic Prostate Score (GPS),[15,16] Cell Cycle Progression Score (CCPS),[17] and Genomic Classifier (GC),[18-22] measure the altered expression of mostly non-overlapping sets of genes that have not been demonstrated to play a direct role associated with the biological events of prostate cancer progression and metastasis. The accuracy of these tests was improved by the addition of clinical and pathological predictors, both as univariate predictors or as captured by the Cancer of the Prostate Risk Assessment (CAPRA-S) score,[23,24] and the Stephenson nomogram.[25] This comparison is limited to post-surgical assessment, because literature review failed to identify studies that provided AUCs or C-indices for pre-surgical assessment. Because expression profiles and CNA analysis are complementary to each other, the accuracy of prediction might be further improved by combining these tests.

When combined with preoperative PSA, MPS could improve the clinical management of men with prostate cancer. Men with early-stage disease and high-risk profiles might benefit from surgery.[26] Men with early-stage disease and low-risk profiles would be candidates for active surveillance that might safely preserve quality of life. Men with higher-risk disease might benefit from adjuvant radiation therapy after surgery.[27] Thus, predicting outcome at the time of diagnosis could affect management for all of these patients.

Tumor heterogeneity was observed in the current study (2/22 matched pairs) and could affect the accuracy of MPS as a predictor (Fig S1). Although multiclonal analysis represents a possible remedy, it will not be available for the vast majority of men with prostate cancer who undergo multicore biopsies and have only a single positive core. Sonographic guidance and serial biopsies could improve the accuracy of prediction, especially for men with early stage disease, where a biopsy with a high MPS score could serve as a trigger for intervention. Future studies will assess the accuracy of MPS derived from biopsies for predicting outcome.

FIGURE LEGENDS

Fig 1. Receiver operating characteristic curves estimating the accuracy of the MPS, preoperative PSA, percent genomic instability, and combined MPS and preoperative PSA to predict mPT and iPT status in both the MSK and Duke cohorts. The AUC is indicated for each curve.

Fig. 2. MPS is associated with metastasis and BCR. Kaplan-Meier plots for (A) metastasis free probability for both cohorts (B) biochemical recurrence free probability for both cohorts. Strata with MPS greater than or equal to (red) or less than (blue) 1.2 in the cohorts are shown. The log-rank significance value is shown for each.

SUPPLEMENTAL FIGURE LEGENDS

Fig. S1. Scatter plot to observe tumor heterogeneity in iPTs (blue) and in mPTs (red). Y-axis represents sorted delta MPS and X-axis represents number of samples.

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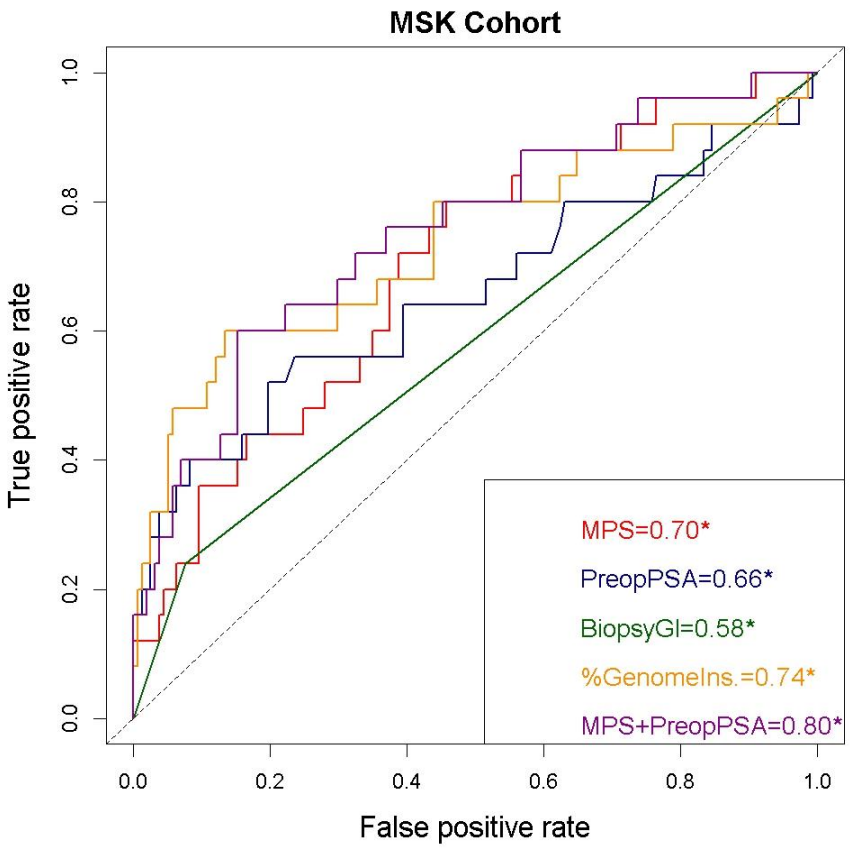
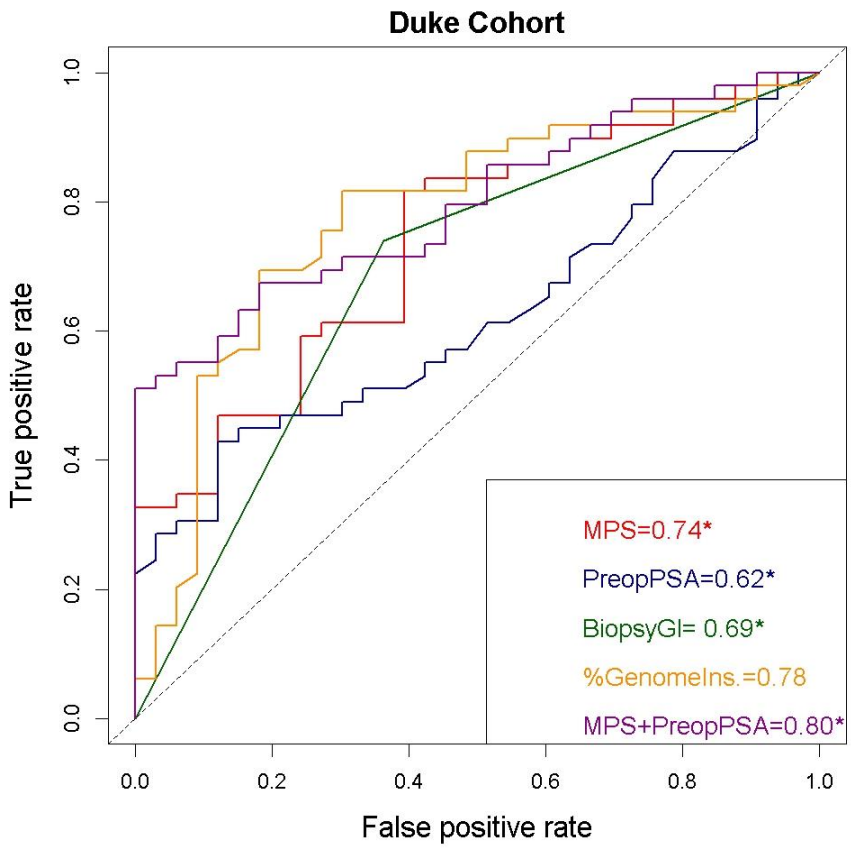
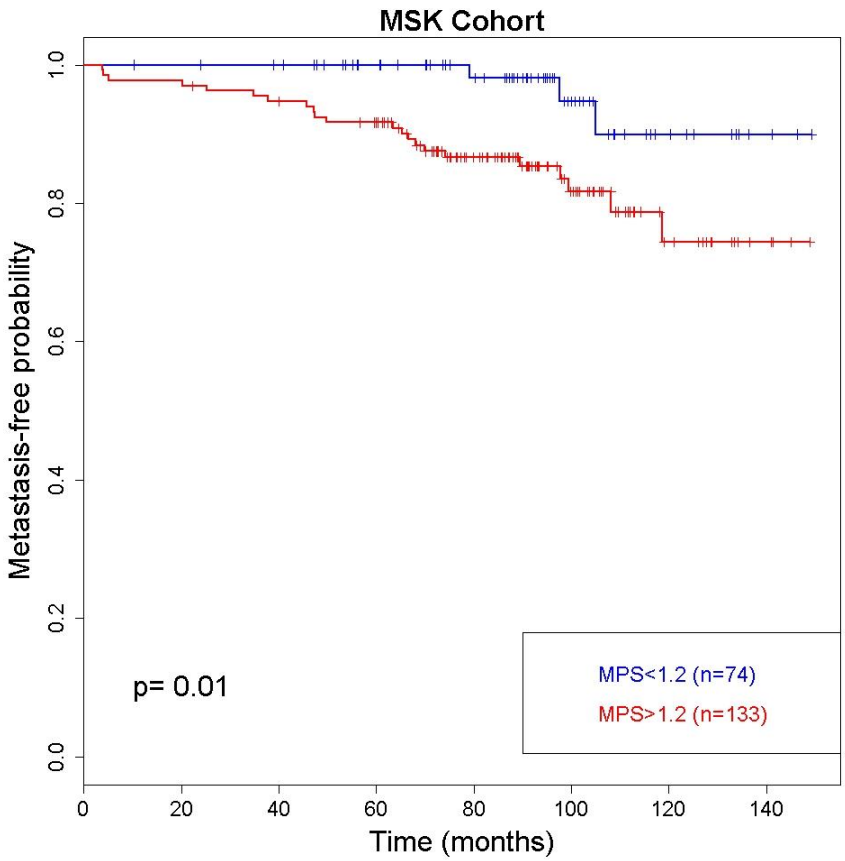
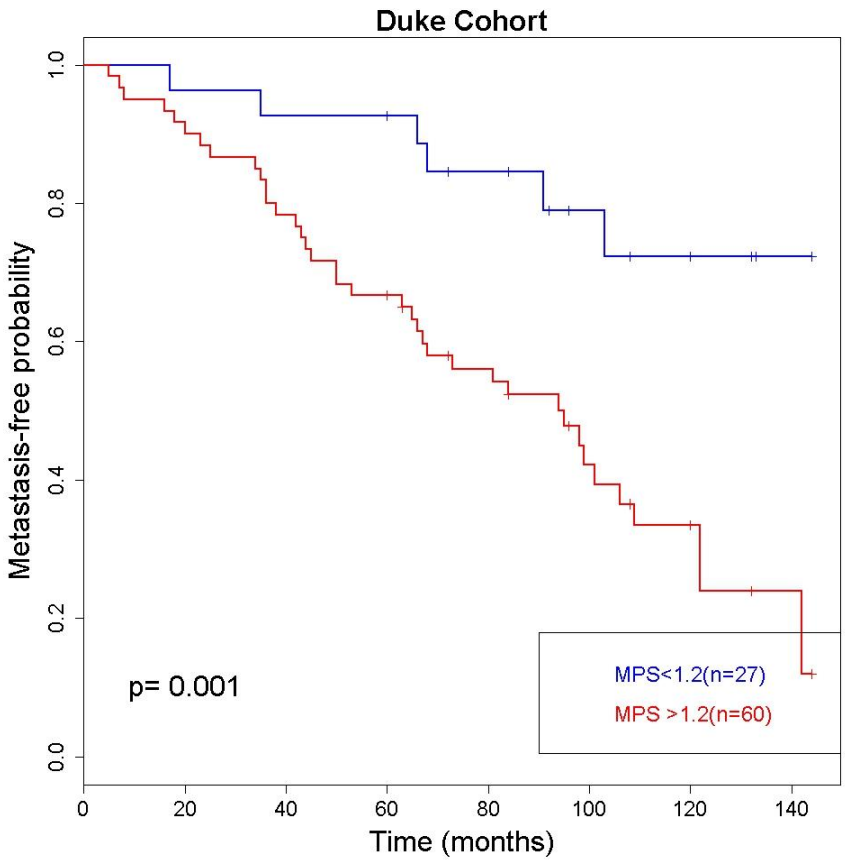
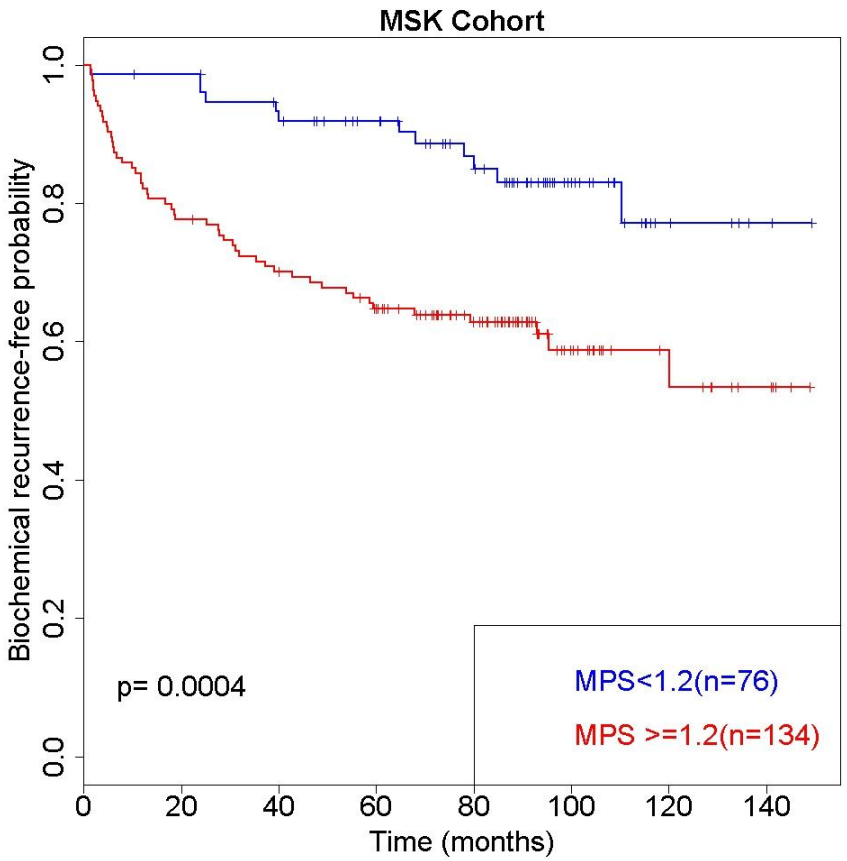

MSK Cohort
True positive rate
False positive rate
0.0
0.2
0.4
0.6
0.8
1.0
MPS=0.70*
PreopPSA=0.66*
BiopsyGl=0.58*
%GenomeIns.=0.74*
MPS+PreopPSA=0.80*


Duke Cohort
True positive rate
False positive rate
0.0
0.2
0.4
0.6
0.8
1.0
MPS=0.74*
PreopPSA=0.62*
BiopsyGl= 0.69*
%GenomeIns.=0.78
MPS+PreopPSA=0.80*


MSK Cohort
Metastasis-free probability
0.0
0.2
0.4
0.6
0.8
1.0
0
20
40
60
80
100
120
140
Time (months)
p= 0.01
MPS<1.2 (n=74)
MPS>1.2 (n=133)


Duke Cohort
Metastasis-free probability
0.0
0.2
0.4
0.6
0.8
1.0
0
20
40
60
80
100
120
140
Time (months)
p= 0.001
MPS<1.2(n=27)
MPS >1.2(n=60)


MSK Cohort
Biochemical recurrence-free probability
1.0
0.8
0.6
0.4
0.2
0.0
0
20
40
60
80
100
120
140
Time (months)
p= 0.0004
MPS<1.2(n=76)
MPS >=1.2(n=134)

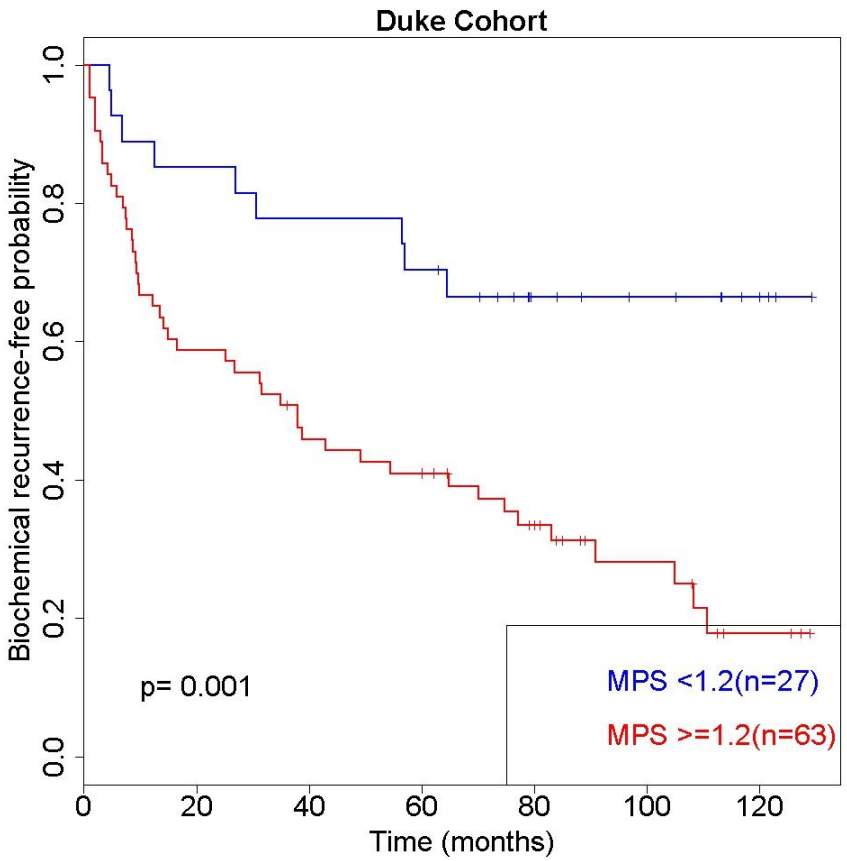

Duke Cohort
Biochemical recurrence-free probability
1.0
0.8
0.6
0.4
0.2
0.0
0
20
40
60
80
100
120
Time (months)
p= 0.001
MPS <1.2(n=27)
MPS >=1.2(n=63)

Table 1. Logistic Regression Model Predicting Progression to Metastasis

Cohort	MSK (n= 182 , mPT=25, iPT=157)				Duke (n= 61, mPT =37, iPT=24)			
Component	AUC	Odds Ratio	P	95% CI	AUC	Odds Ratio	P	95% CI
Univariate								
MPS	0.70	5.98	0.001	2.12 to 18.57	0.75	11.84	0.002	2.78 to 67.71
Pre-operative PSA	0.66	1.06	0.01	1.02 to 1.11	0.61	1.1	0.08	1.01 to 1.21
Multivariate								
MPS	0.80	5.58	0.04	1.20 to 32.90	0.80	16.85	0.001	3.43 to 118.67
Pre-operative PSA		1.05	0.06	1.01 to 1.12		1.1	0.05	1.01 to 1.25

Table 2. Logistic Regression Model Predicting Biochemical Recurrence

Cohort	MSK (n= 222, BCR+ =65, BCR- =157)				Duke (n= 76 , BCR+ =37, BCR- =39)			
Component	AUC	Odds Ratio	P	95% CI	AUC	Odds Ratio	P	95% CI
Univariate								
MPS	0.67	4.51	5.50E-05	2.22 to 9.64	0.70	6.92	0.003	2.02 to 28.09
Pre-operative PSA	0.67	1.08	0.0001	1.04 to 1.13	0.55	1.02	0.29	0.98 to 1.09
Multivariate								
MPS	0.73	3.1	0.004	1.45 to 6.91	0.71	7	0.003	2.03 to 28.43
Pre-operative PSA		1.06	0.001	1.03 to 1.12		1.03	0.28	0.98 to 1.1

Table 3. Cox Proportional Hazards Model of MPS and its Association With Metastasis-Free Survival

Cohort	MSK (n= 222 , mPT=25, iPT=197)					Duke (n= 76 , mPT=37, iPT=39)				
Component	Hazard Ratio	P	95% CI	Conc-indx	P	Hazard Ratio	P	95% CI	Conc-indx	P
Univariate										
MPS	4.2	0.002	1.67 to 10.36	0.70	6.00E-05	3.9	0.01	1.48 to 10.43	0.63	0.01
Pre-operative PSA	1.01	7.60E-05	1.00 to 1.01	0.63	0.08	1.00	0.98	0.97 to 1.03	0.51	0.94
Multivariate										
MPS	4.06	0.003	1.60 to 10.34	0.74	1.30E-06	3.7	0.03	1.15 to 11.72	0.70	8E-04
Pre-operative PSA	1.01	0.0002	1.00 to 1.01			1.1	0.01	1.02 to 1.12		

Table 4. Cox Proportional Hazards Model of MPS and its Association With Biochemical Recurrence

Cohort	MSK (n= 222, BCR+ =65, BCR- =157)					Duke (n= 76 , BCR+ =37, BCR- =39)				
Component	Hazard Ratio	P	95% CI	Conc-indx	P	Hazard Ratio	P	95% CI	Conc-indx	P
Univariate										
MPS	3.5	1.50E-05	1.97 to 6.50	0.67	1.12E-07	3.5	0.002	1.6 to 7.7	0.62	0.004
Pre-operative PSA	1.01	1.00E-04	1.00 to 1.01	0.66	2.80E-05	1.02	0.15	0.99 to 1.1	0.54	0.40
Multivariate										
MPS	3.40	2.90E-05	1.9 to 5.91	0.69	1.60E-09	3.6	0	1.6 to 8.2	0.51	0.82
Pre-operative PSA	1.01	0.001	1.00 to 1.01			1.0	0.1	0.99 to 1.05		